flow, was mildly increased at 1 hr (2.0±2.3), then increased over the first week peaking at day 4 (5.6±2.6), then declined rapidly (0.9±0.7 at day 28). The MB_E signal peak preceded increases in normalized blood flow and blood volume, were not detected until after day 14 (0.52±0.11 and 0.87±0.09, for blood flow and volume at 28 days). MB_E signal peak also preceded an increase in ischemic muscle tissue PO_2 (normalized values of 0.24±0.06 and 0.47±0.10, at 1 hr and 28 days, respectively).
We conclude that CEU with microbubbles targeted for endothelial α_v-integrins can be used to non-invasively assess angiogenic responses in skeletal muscle. These results suggest that targeted CEU imaging of endothelial markers of angiogenesis may potentially be used for assessing intrinsic and therapeutic angiogenesis prior to changes in perfusion.

9:30 a.m.

802-2

Increased Suppression of Intracoronary C-myc Protein Synthesis Within the Stent or Balloon Injury Site Using an Intravenous Microbubble Delivery System Containing Antisense to C-myc: Comparison With Direct Intracoronary Injection

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BACKGROUND: Although perfluorocarbon containing albumin microbubbles (PESDA) can bind large quantities of antisense (AS) to the c-myc protooncogene (anti-c-myc) which promotes intimal hyperplasia, it is unknown how much c-myc synthesis within the intracoronary (IC) stent or balloon injury is actually suppressed by this intravenous (IV) targeting technique in the early period following vascular injury. To examine this, we performed high phase liquid chromatography of AS to c-myc uptake and Western Blot studies of c-myc protein synthesis in coronary arteries from eight pigs 90 minutes following IC stent and balloon injury (two vessels per pig). Pigs were treated with either direct IC anti-c-myc (4 milligrams), or the same dose of anti-c-myc IV bound or unbound to PESDA. IV PESDA containing anti-c-myc was given in the presence or absence of transthoracic 1 megahertz ultrasound (TTU) (pulsed wave at 0.6 W/cm^2). **RESULTS:** C-myc protein synthesis in the injured coronary arteries (normalized for control vessels) was significantly lower when pigs were given IV anti-c-myc bound to PESDA irrespective of whether TTU was concomitantly delivered (TABLE). Suppression of c-myc synthesis was comparable to direct IC injection. **CONCLUSION:** These data confirm that simply binding anti-c-myc to IV PESDA is a non-invasive method of targeting therapeutic genes to selective sites of IC balloon or stent injury and suppressing the formation of the c-myc protooncogene which mediates intimal hyperplasia and restenosis.

*p<0.05 compared to other groups (ANOVA)

	Direct IC AS	IV AS/PESDA	IV AS/PESDA + TTU	IV AS + TTU
c-myc protein ratio	0.94±0.26	0.88±0.11	0.89±0.28	2.11±0.28*
anti-c-myc Uptake (nanograms)	13±16	24±3	31±6	29±38

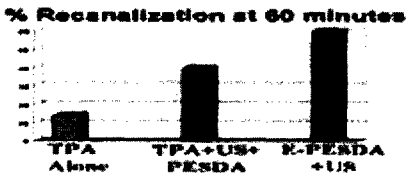
9:45 a.m.

802-3

Improvement in Epicardial Recanalization Rates With Transthoracic Therapeutic Ultrasound and Intravenous Microbubbles Containing Ligands Which Attach to the Glycoprotein IIb/IIIa Receptor on Activated Platelets

Thomas R. Porter, Joseph Oberdorfer, Lucia Venneri, John Lof, Feng Xie, University of Nebraska Medical Center, Omaha, NE

BACKGROUND: Intravenous (IV) perfluorocarbon containing microbubbles (PCMB) and transthoracic ultrasound (TTU) could potentially be used to improve epicardial recanalization rates (ERR) following acute coronary thrombotic occlusion. One limitation with this method is that inadequate PCMB reach the occluded coronary thrombus (CT) following IV injection. We hypothesized that attaching a ligand to the PCMB which binds to activated platelets within the CT would increase microbubble concentration at the site of the CT and enhance cavitation-induced lysis. Accordingly, we compared ERR following acute left circumflex thrombotic occlusion in 50 pigs who were randomized to receive either IV tissue plasminogen activator (TPA) alone, versus IV TPA in combination with TTU and unlabelled IV PCMB (PESDA); or TTU with IV PCMB containing 50 u/kg of Eptifabitide (E-PESDA). TTU was intermittent 1 Megahertz at 1.5 Watts/cm^2. Recanalization was assessed angiographically at 60 minutes following treatment. **RESULTS:** Activated clotting times were not different following any of the treatments. However, the Figure demonstrates that ERR increased from <20% with TPA alone to 60% with IV TPA and IV E-PESDA combined with TTU. **CONCLUSION:** These data indicate that IV E-PESDA can be used to increase delivery of microbubbles to acute coronary thrombotic occlusions and improve ultrasound-directed recanalization of the infarct related artery.



10:00 a.m.

802-4

Site-Specific Imaging of Tumor Angiogenesis Using Contrast-Enhanced Ultrasound Imaging With Microbubbles Targeted to Alpha-V Beta-3

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Background: Identification of tumor angiogenesis could potentially be used for early diagnosis of neoplasms and for prognosis. We hypothesized that contrast-enhanced ultrasound (CEU) with microbubbles targeted to endothelial $\alpha_v\beta_3$ expressed in neovessels could be used to assess tumor angiogenesis.
Methods: We created a brain tumor model where 10^5 U87MG cells derived from a human glioblastoma tumor cell line were embedded in gelfoam and injected intracerebrally in athymic rats. Tumors were assessed after either 14 or 28 days of growth (n=4 for each). Control rats (n=4) were injected with gelfoam alone. Targeted CEU imaging was performed 15 min following i.v. injection of control microbubbles (MB) or $\alpha_v\beta_3$ targeted microbubbles (MB_E) bearing the disintegrin echistatin on their surface. Cerebral perfusion was assessed by CEU during continuous infusion of non-targeted microbubbles. A corresponding brain slice was processed for immunohistochemistry.
Results: Tumors were ~4-fold larger at day 28 compared to day 14 ($p<0.05$). Perfusion was detected within all tumors and was characterized by low microvascular blood velocity. On histology, 15-50 μm neovessels were abundant within the tumor and stained positive for endothelial PECAM-1. Dense staining for $\alpha_v\beta_3$ on the endothelium was found within tumors, especially at the outer margins, whereas minimal staining was seen in control regions. On CEU, MB_E signal intensity in gelfoam injection sites was low and similar to that in the contralateral hemisphere. At both 14 and 28 days, MB_E signal in tumors was significantly ($p<0.01$) greater than that for MB_E in normal control regions, or for MB within the tumor. MB_E signal in tumors was greater at 28 versus 14 days ($p<0.05$), and was greatest at the peripheral margins of the tumors. MB_E intensity within tumors correlated well with microvascular blood volume derived from CEU perfusion imaging ($r=0.83$, $p<0.01$).
Conclusions: CEU with microbubbles targeted for endothelial $\alpha_v\beta_3$ can be used to assess tumor angiogenesis. These results have important implications for developing methods for early detection of primary or metastatic disease, or for developing novel anti-neoplastic therapies with microbubble delivery systems.

10:15 a.m.

802-5

Ultrasound Targeted Microbubble Destruction Can Direct Adenoviral or Plasmid Gene Expression to the Heart, Pancreas, and Brain

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We have previously shown that ultrasound targeted microbubble destruction (UTMD) can augment expression of an adenoviral reporter in the heart. We now show that this method can selectively deliver transgenes to two organs that are particularly inaccessible to non-invasive strategies (pancreas and brain) and can be extended to plasmid vectors. Recombinant adenoviruses or plasmids containing expression constructs of beta-galactosidase and luciferase were incorporated into albumin-coated perfluoropropane-filled microbubbles during their preparation. These bubbles were infused into the internal jugular vein of rats and destroyed with ultrasound while passing through the target organ. Organs were harvested after 4 days and analyzed for reporter gene activity. Luciferase activity in organs targeted with adenovirus was 104 times higher than in control organs. However, liver activity was even higher. Histological examination revealed transgene-derived beta-galactosidase activity in subsets of brain neurons and pancreatic islets. Luciferase transfection with plasmids showed highly specific gene expression in the heart, 10-fold lower than obtained with adenovirus, but with negligible activity in liver (figure). We conclude that ultrasound targeted microbubble destruction can markedly improve the range and specificity of gene delivery. This technique heralds a new class of strategies for non-invasive gene therapy.